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Anxiolytic effects of diphenyl diselenide on adult zebrafish in a novelty paradigm

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ABSTRACT

Anxiety-related disorders are frequently observed in the population. Because the available pharmacotherapies for anxiety can cause side effects, new anxiolytic compounds have been screened using behavioral tasks. For example, diphenyl diselenide $(PhSe)_2$, a simple organoselenium compound with neuroprotective effects, has demonstrated anxiolytic effects in rodents. However, this compound has not yet been tested in a novelty-based paradigm in non-mammalian animal models. In this study, we assessed the potential anxiolytic effects of $(PhSe)_2$ on the behavior of adult zebrafish under novelty-induced stress. The animals were pretreated with 0.1, 0.25, 0.5, and 1 μM $(PhSe)_2$ in the aquarium water for 30 min. The fish were then exposed to a novel tank, and their behavior was quantified during a 6-min trial. $(PhSe)_2$ treatment altered fish behavior in a concentration-dependent manner. At 0.01 and 0.25 μM, $(PhSe)_2$ did not elicit effects on fish behavior. At 0.5 μM, moderate behavioral side effects (e.g., lethargy and short episodic immobility) were noted. At the highest concentration tested (1 μM), dramatic side effects were observed, such as burst behavior and longer periods of immobility. The results were confirmed by spatiotemporal analysis of each group. Occupancy plot data showed dispersed homebase formation in the 0.25 μM $(PhSe)_2$-treated group compared with the control group (treated with 0.04% DMSO). Furthermore, animals treated with 0.25 μM $(PhSe)_2$ showed a reduction in latency to enter the top and spent more time in the upper area of the tank. These data suggest that $(PhSe)_2$ may induce an anxiolytic-like effect in situations of anxiety evoked by novelty.

1. Introduction

Anxiety-related disorders are the most prevalent disorders in the world population (Anon., 2002). This type of psychiatric condition is usually a comorbidity associated with a large number of another disorders of the brain (Brooks-Kayal et al., 2013; Caruso et al., 2013; Cheung, 2013), and anxiety involves common neurological circuits (De Masi, 2004). Considering that the available pharmacotherapies for anxiety may cause several side effects, such as sedation, muscle relaxation, amnesia, and dependence, new drugs with few undesirable effects have been screened as candidates for the treatment of anxiety disorders (Otto et al., 2010; Youssef and Rich, 2008).

Novelty is defined as a new or unfamiliar experience. Due to the lack of experience in the new situations, the brain identifies novel situations as stressful moments that induce anxiety-like behaviors. In basic neuroscience research, the novel tank diving test (or the open tank paradigm) is an emergent behavioral tool that is used to measure novelty-associated behavioral stress responses. Fundamentally, this task consists of assessing the vertical exploratory activity of zebrafish in a new environment. The task is based on the initial tendency of the fish to dive to the bottom and gradually swim to the upper areas of the new tank (Levin et al., 2007). As demonstrated by Stewart et al. (2011), this test can be used for investigating pharmacological

Abbreviations: $(PhSe)_2$, Diphenyl diselenide.

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modulators of anxiety-like phenotypes in adult zebrafish. Moreover, the novel tank test can be used to assess the complete exploratory and locomotor behavior of zebrafish (Rosemberg et al., 2011). This perspective allows for the assessment of the potential toxic effects of classical anxiolytic drugs, such as benzodiazepines (Bencan et al., 2009), as well as substances that promote anxiolytic-like effects at low doses and sedation at higher concentrations (Rosemberg et al., 2012).

In the last several years, many compounds that have demonstrated anxiolytic effects in rodents have emerged. In this context, interest in organoselenium compounds has increased because they have anxiolytic-like (Savegnago et al., 2008), antimicrobial (Kumar et al., 2010), antineoplastic (Micke et al., 2010) and anti-inflammatory (Duntas, 2009) properties. One interesting selenium-containing molecule is diphenyl diselenide $(PhSe)_2$, which has known pharmacological effects in rodents (Luchese et al., 2007, 2009; Nogueira and Rocha, 2010; Nogueira et al., 2004; Prigol et al., 2009; Savegnago et al., 2007) and shows anxiolytic-like effects in the open-field and plus-maze tests (Ghisleni et al., 2008). In chickens, the anxiolytic-like effects of $(PhSe)_2$ were observed after social separation-stress behavior (Prigol et al., 2011). Despite the extensive literature describing the anxiolytic effects of $(PhSe)_2$, it is difficult to validate putative molecular mechanisms that predict toxic effects in mammals. Consequently, the study of the behavioral effects of diphenyl diselenide in an alternative and simple animal model is of particular importance. Importantly, aquatic vertebrates have more selenoproteins than terrestrial organisms, and $(PhSe)_2$ can partially mimic the activity of selenoproteins (Mariotti et al., 2012; Nogueira and Rocha, 2010). Furthermore, teleosts have a narrower range of tolerance to selenium, and the use of the zebrafish model may serve as a primary screening step to investigate the potential toxicological effects of organo-chalcogen compounds, complementing the existing rodent approaches. One interesting method for analyzing the potential pharmacological and toxicological effects of new compounds is the measurement of the behavioral repertoire of the organism. Animal behavior involves the interaction of an organism with the surrounding environment. In fact, several behavioral parameters may be used to predict both the anxiolytic and toxic effects of chemical substances. Because adult zebrafish display a wide range of behaviors that have previously been pharmacologically characterized (Kalueff et al., 2013; Maximino et al., 2011; Rosemberg et al., 2012), the assessment of the behavioral phenotypes of this species may also reveal the primary effects of diphenyl diselenide in fish. Furthermore, because anxiety can be elicited by various situations in humans, distinct behavioral tasks and animal models may be used to indicate whether $(PhSe)_2$ maintains its anxiolytic effects in various anxiogenic situations.

To assess whether $(PhSe)_2$ induces anxiolytic-like behavior in a stressful condition such as novelty, we used the novel tank paradigm after acute exposure of zebrafish to $(PhSe)_2$. Moreover, because selenium compounds have a narrow concentration range between beneficial and toxicological effects, we also performed a thorough screening of fish behavior to examine the possible behavioral side effects of $(PhSe)_2$.

2. Materials and methods

2.1. Animals

Adult zebrafish (*Danio rerio*; 4 to 6 months old, approximately 50:50 male:female ratio) from a heterogeneous wild-type stock (standard short-fin phenotype) were obtained from a local commercial supplier (Delphis, RS, Brazil). The fish were housed in 50-L aquariums (80–100 fish per aquarium) for at least 2 weeks prior to the experiments to allow them to acclimate to the animal facility. All tanks were filled with non-chlorinated water previously treated with 132 μL/L AquaSafe (Tetra, VA, USA) and maintained with mechanical and chemical filtration at a target temperature of 26 ± 2 °C and a water pH of 7.0 to 8.0 (system water). The room illumination was provided by ceiling-mounted fluorescent lamps on a 14/10 light/dark photoperiod (lights on at 7:00 a.m.). The animals were fed twice a day with a commercial flake fish food (Alcon BASIC, Alcon, Brazil) and maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011). All procedures with animal subjects were approved by the Ethics Committee for the Use of Animals—CEUA from the Universidade Federal do Rio Grande do Sul. Due to the conditions of protocol approval, maximum efforts were made to minimize the number of animals used and their suffering.

2.2. Treatments with $(PhSe)_2$ and behavioral analysis

Fish ($n = 12$ in each group) were individually treated with $(PhSe)_2$ (0.1, 2.5, 0.5, or 1 μM) for 30 min. The $(PhSe)_2$ was prepared in DMSO and then diluted in water to the above concentrations (the final concentration of DMSO in all solutions of $(PhSe)_2$ was adjusted to 0.04%). The control group was exposed to system water containing 0.04% DMSO. After 30 min of exposure, the animals were carefully placed individually in a trapezoidal tank (23.9 cm along the bottom × 28.9 cm at the top, 15.1 cm in height) filled with home system water. To maintain the same experimental conditions, all of the experiments were performed during the same time period each day (from 10:00 a.m. to 4:00 p.m.). The fish were randomly handled, removed from their home tanks, and individually transferred to beakers filled with the solutions. For each experiment, a single fish was placed in each solution, and the solution was not used in subsequent experiments.

A webcam (Microsoft LifeCam 1.1 with Auto-Focus) was placed in front of the novel tank to monitor the location and swimming activity of the zebrafish during the 6-min trial. To ensure a uniform background for the video analysis and to avoid environmental distraction for animals subjected to the behavioral test, yellow sheets of paper were placed 4.3 cm behind the tank and also on both sides of the apparatus. Two 60-W light bulbs were placed 40 cm behind the novel tank to increase the contrast between the background and the fish. The webcam was connected to a laptop for recording the videos. The behavioral parameters were automatically measured at a rate of 30 frames/s using appropriate video-tracking software (ANY-maze, Stoelting CO, USA). During all of the experiments, care was taken to move fish gently between home tanks, beakers, and the novel tank to avoid handling stress. Each experimental group comprised individuals from multiple batches, and the tank water was replaced with clean system water for individual trials. All fish were handled and tested using standardized procedures (similar manipulation, water quality, and illumination).

2.3. Spatiotemporal analysis of behavior

To design representative ethograms of the $(PhSe)_2$-exposed groups, the behavioral profiles of zebrafish in the novel tank were analyzed using the track reconstructions of the spatial coordinates over time (Cachat et al., 2011; Grossman et al., 2010; Rosemberg et al., 2011). Briefly, the coordinates of the experimental tank were properly calibrated using the ANY-maze software, and the tracking data across fractions of a second for each fish was exported as raw data into separate spreadsheets. The exported spatial coordinates (x-center and y-center) were evaluated based on their similarity to each other by two trained observers (inter-rater reliability >0.85) on a consensus basis. The middle trace was selected as representative of the group to illustrate the spatiotemporal pattern of exploration. Spatiotemporal reconstructions were represented as scatter plots, which were constructed using Graphis 3D graphing software. The x-center (horizontal distribution), y-center (vertical distribution), and time (z-axis) were plotted. The positions of the fish across the trial were represented using a spectrum of colors (blue–red) to demonstrate the position of the animal during the test (0–360 s).

2.4. Locomotor parameters, vertical exploration and homebase formation

The locomotor activity of zebrafish was measured using the following behavioral endpoints: 1) the total distance traveled; 2) the time of immobility; and 3) the maximum speed. Additionally, the maximum speed and immobility time were determined in constant time intervals (5 s) to quantify the pattern of behavioral changes within the trial. The vertical behavior of a zebrafish in the novel tank represents its tendency to gradually explore upper areas when exposed to a novel apparatus, which may reflect habituation to the new environment (Rosemberg et al., 2011; Wong et al., 2010). Moreover, vertical activity (e.g., time spent at the top of the tank) has been suggested as an indicator of reduced anxiety levels (Egan et al., 2009; Levin et al, 2007; Mathur and Guo, 2011). The exploratory profile of fish was estimated by quantifying the horizontal and vertical parameters as described by Rosemberg et al (2011). We evaluated homebase formation during the novel tank trial, which is defined as the location in the tank that is preferred by adult zebrafish over time (Stewart et al., 2010a,b). In summary, the novel tank was divided into three areas (bottom, middle and top). Each of the areas was subdivided into five sections. The occupancy plot, represented as a heat graph (blue to red), indicates the time that the animals spent in each section. The homebase is represented as the section where the animals spent the most time, interpreted as a "safe" place for exploration. If the group shows replicable behavior (i.e., no large inter-individual variation) when all the animals are plotted in a single occupancy plot, variation in color (yellow to red) is observed (Rosemberg et al., 2011, 2012).

2.5. Statistics

Parametric data were expressed as the means ± standard error of the mean (S.E.M.) and analyzed by repeated-measures analysis of variance (ANOVA) using Bonferroni's post hoc test. The results of temporal analyses of maximum speed and immobility were expressed as the mean ± S.E.M. and analyzed by two-way ANOVA followed by a multiple comparison test. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

Animals treated with anxiolytic drugs can exhibit lethargy or episodes of immobility as side effects. Because there are no studies describing the effects of $(PhSe)_2$ in zebrafish, we first tested this compound at concentrations ranging from 0.1 to 1 μM. Animals exposed to lower concentrations of $(PhSe)_2$ (0.1 and 0.25 μM) did not show changes in distance traveled compared to the control fish. However, fish exposed to higher concentrations (0.5 μM and 1 μM) showed a significant reduction in this parameter (one-way ANOVA, $F_{[4,59]} = 33.16$, $p < 0.0001$; Bonferroni test, $p < 0.05$ for both concentrations, respectively) (Fig. 1A). Animals exposed to 1 μM $(PhSe)_2$ spent more time immobile (Fig. 1B) and had the highest maximum speed (Fig. 1C) of all groups (one-way ANOVA, $F_{[4,59]} = 4.606$, $p < 0.001$; Bonferroni test, $p < 0.05$; one-way ANOVA, $F_{[4,59]} = 10.55$, $p < 0.0001$; Bonferroni test, $p < 0.05$, respectively). To isolate the effects of 0.5 and 1 μM $(PhSe)_2$, we also performed an analysis of maximum speed over short periods of time (5 s). Animals exposed to 0.5 μM $(PhSe)_2$ displayed a constant maximum speed throughout the trial. However, the group exposed to 1 μM $(PhSe)_2$ showed several peaks of maximum speed, indicating the occurrence of burst swimming (Fig. 2A) (two-way ANOVA, $F_{[1,22]} = 15.90$, $p < 0.0001$ for both concentrations). Because these peaks of maximum speed occurred mostly after the first minute and anxiolytic drugs can evoke immobility in zebrafish immediately following the removal of the drug (Mussulini et al., 2013), we further analyzed the time of immobility during the trial. Fig. 2B shows increased immobility in fish exposed to 1 μM $(PhSe)_2$, with recurrent episodes of immobility during the trial (two-way ANOVA, $F_{[4,49]} = 4.583$, $p < 0.001$; multiple comparison test, $p < 0.01$). Thus, the effects of $(PhSe)_2$ on zebrafish behavior were concentration-dependent. Indeed, low concentrations of $(PhSe)_2$ (0.01 and 0.25 μM) caused no behavioral side effects. An intermediate concentration (0.5 μM) caused moderate side effects, such as lethargy (decreased distance and an equal duration of immobility when compared to the control) and brief instances of immobility, and the highest concentration (1 μM) caused drastic side effects, such as burst swimming and a prolonged period of immobility, with possible toxicity.

We next assessed whether $(PhSe)_2$ could induce anxiolytic-like effects during a novelty stress condition using representative spatio-temporal analysis of zebrafish behavior. Importantly, the behaviors of the control group (exposed to 0.04% DMSO) were similar to those observed in untreated fish, which we have previously described (Rosemberg et al., 2011, 2012). The animals showed a preference for the bottom area, whereas the middle was primarily used for vertical transitions, in which animals demonstrated low horizontal exploration (Fig. 3A). The group exposed to 0.1 μM $(PhSe)_2$ showed an overall behavioral profile similar to that of the control. This group used the middle area as a passage to the top and bottom areas, but animals rapidly approached the top at the beginning of the test (Fig. 3B). The group treated with 0.25 μM $(PhSe)_2$ showed a different profile, in which the animals demonstrated initial exploration of the bottom with fast access to all vertical areas. In the final 3 min of the test, the fish in this group changed their exploratory activity, showing a preference for exploring the top area of the tank (Fig. 3C). The group treated with 0.5 μM $(PhSe)_2$ showed chaotic behavior. These animals displayed fast transitions to the top and bottom and spent more time in the middle area of the apparatus (Fig. 3D). Fish exposed to 1 μM $(PhSe)_2$ initially showed a longer period of immobility at the bottom of the apparatus. Later, the animals approached the top of the apparatus and showed

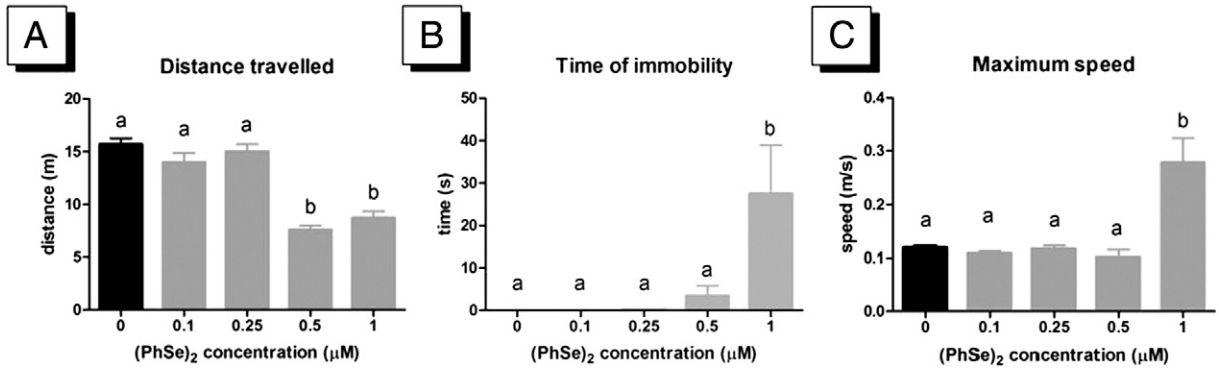

Fig. 1. Basic endpoint behaviors used to assess the proper $(PhSe)_2$ concentration range for adult zebrafish. The graph shows the distance traveled (A), time of immobility (B) and maximum speed (C). The data were analyzed by one-way ANOVA followed by Bonferroni's post hoc test, considering $p \leq 0.05$ as significant. Different letters indicate significant differences among the control (black) and $(PhSe)_2$ groups (gray).

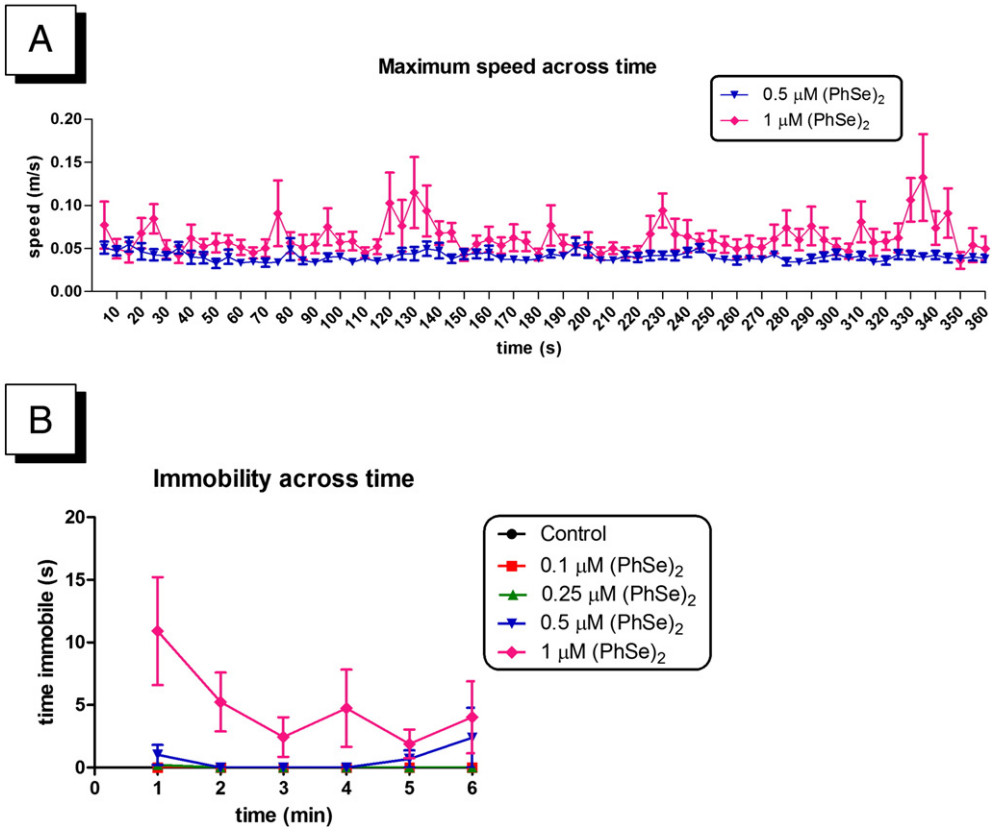

Fig. 2. Analysis of possible toxicological effects on adult zebrafish behavior. Maximum speed over time (A) of fish exposed to 0.5 μM (-▼-) and 1 μM (-◆-) $(PhSe)_2$. Peaks indicate burst behavior with a significant difference between groups (two-way ANOVA, $p < 0.0001$). Immobility over time (B) compared among all groups. Two-way ANOVA, $p < 0.001$, followed by a multiple comparison test, $p < 0.01$, indicates that only fish treated with 1 μM $(PhSe)_2$ differed from those in all other groups.

stereotypical behaviors, alternating between short episodes of immobility and burst swimming (Fig. 3E).

The occupancy plot analyses showed a progressive decrease in homebase formation. The control group demonstrated a clear homebase, with a continuous return to the same location in the bottom area (Fig. 4A). Animals treated with 0.1 μM $(PhSe)_2$ began to form dispersed homebases, returning to distinct safe points during the test (Fig. 4B). The dispersal was more obvious in animals exposed to 0.25 μM $(PhSe)_2$. This group did not form a specific homebase, instead exploring the entire apparatus (Fig. 4C). The occupancy plot analysis confirmed the chaotic behavior demonstrated by the animals treated with 0.5 μM $(PhSe)_2$, which did not have a preference for a particular location in the apparatus (Fig. 4D). The animals exposed to 1 μM $(PhSe)_2$ had two homebases at the top of the apparatus and few exploration areas in the middle (Fig. 4E).

Classical endpoint analysis (Savio et al., 2012) confirmed the behavioral profile described for each group. Animals treated with 1 μM $(PhSe)_2$ showed a significant reduction in the time spent at the bottom (Fig. 5A) (one-way ANOVA, $F_{[4,59]} = 78.30$, $p < 0.0001$; Bonferroni test, $p < 0.05$), whereas fish treated with 0.5 μM $(PhSe)_2$ spent more time in the middle area of the new tank (Fig. 5B) (one-way ANOVA, $F_{[4,59]} = 49.79$, $p < 0.0001$; Bonferroni test, $p < 0.05$). Regarding the time spent at the top, no difference between the control and the 0.1 μM $(PhSe)_2$-treated groups was observed. Animals treated with 0.25 and 1 μM $(PhSe)_2$ spent more time at the top compared with the control group (Fig. 5C) (one-way ANOVA, $F_{[4,59]} = 187.3$, $p < 0.0001$; Bonferroni test, $p < 0.05$). The latency to enter the top showed a clear U-shaped curve, in which the fish groups treated with 0.1, 0.25 and 0.5 μM $(PhSe)_2$ showed significantly lower latencies to enter the top portion of the tank when compared with the control group (Fig. 5D) (one-way ANOVA, $F_{[4,59]} = 30.58$, $p < 0.0001$; Bonferroni test, $p < 0.05$).

4. Discussion

In recent years, the zebrafish has emerged as a useful model to study neuropsychiatric disorders. A recent review by Stewart et al (2012) describes the potential of zebrafish for studying anxiety-like phenotypes from a behavioral perspective using the novel tank diving test. In addition, we have proposed an ethogram for this paradigm, which is also considered a reliable test for evaluating the overall exploratory profile of animals during novelty stress (Rosemberg et al., 2011). Furthermore, the zebrafish model offers new insights for translational neuroscience research (Stewart et al., 2014) and allows for the exploration of complex brain disorders (Kalueff et al., 2014).

Anxiety can be elicited by a vast array of experiences. Currently, there is no specific behavioral trial that can be used to properly characterize anxiolytic drugs. Nevertheless, approaches from various perspectives using different animal models can maximize the identification of more efficient drugs in preliminary screening experiments (Ramos, 2008; Steenbergen et al., 2011). From this perspective, $(PhSe)_2$ has demonstrated a potential anxiolytic-like effect on rodents during behavioral tasks (e.g., a reduction in the number of fecal bolus in the open-field test as well as an increase in the time spent and entries into the open arm of a plus-maze (Ghisleni et al., 2008) and into the lit compartment of a light–dark box (Savegnago et al., 2008). Moreover, chickens treated with $(PhSe)_2$ had low anxiety levels based on a test of their social separation-stress behavior (Prigol et al., 2011). However, the present study is the first to describe the potential anxiolytic-like effects of $(PhSe)_2$ when animals are subjected to novelty stress.

The zebrafish exposed to 0.25 μM $(PhSe)_2$ showed a reduction in the latency to enter the top area of the novel tank. This parameter is an indicator of low anxiety, which was first reported by Levin et al

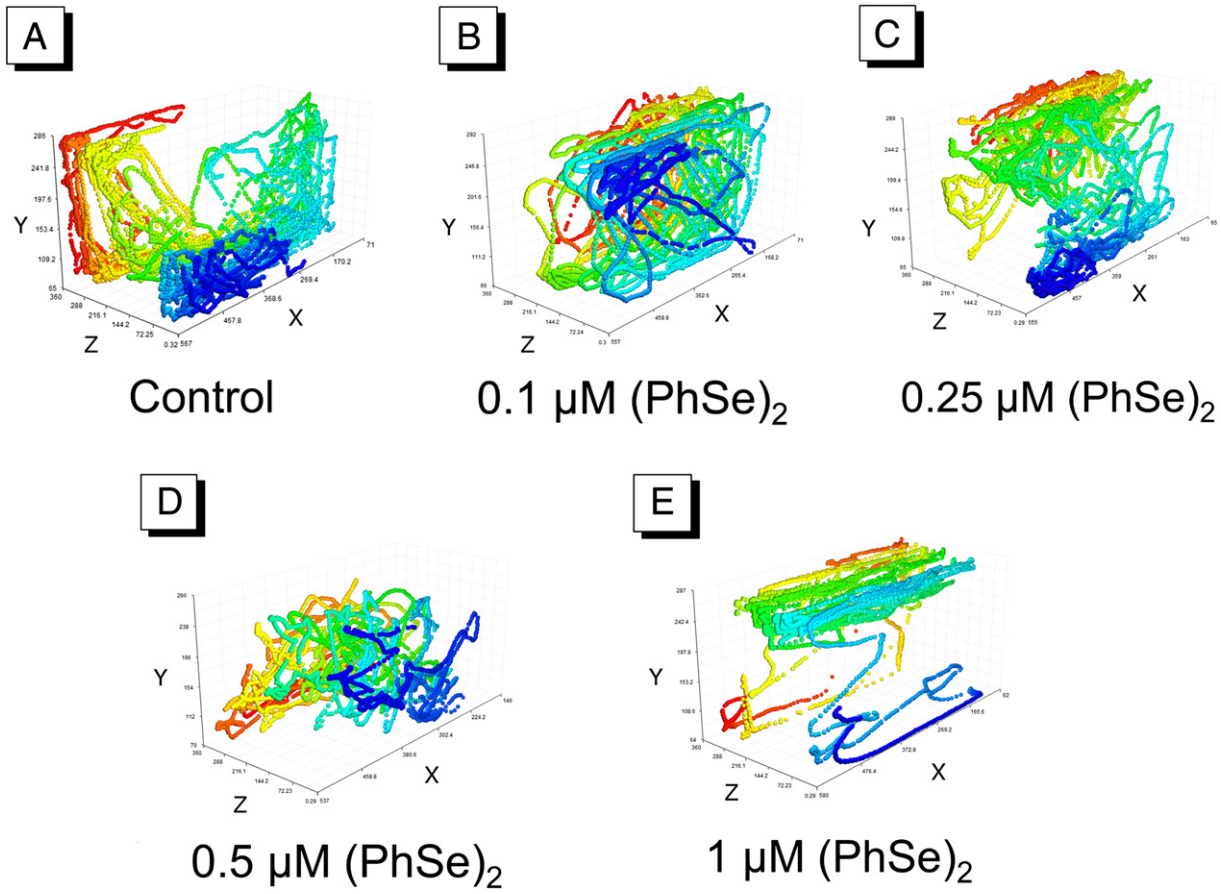

Fig. 3. Spatiotemporal analysis of adult zebrafish exposed to a range of $(PhSe)_2$ concentrations. Representative spatiotemporal reconstructions of fish treated with 0.04% DMSO (control) (A) and 0.1 μM (B), 0.25 μM (C), 0.5 μM (D) and 1 μM $(PhSe)_2$ (E) during the 6 min of the test were obtained by plotting animal traces (X-axis and Y-axis) over time (Z-axis). The test segments (0–360 s) are represented by a color scale gradient and are shown in the Z-axis (blue to red). Dots in the same spot over time indicate immobility. Dots with a large distance over short time periods indicate burst behavior.

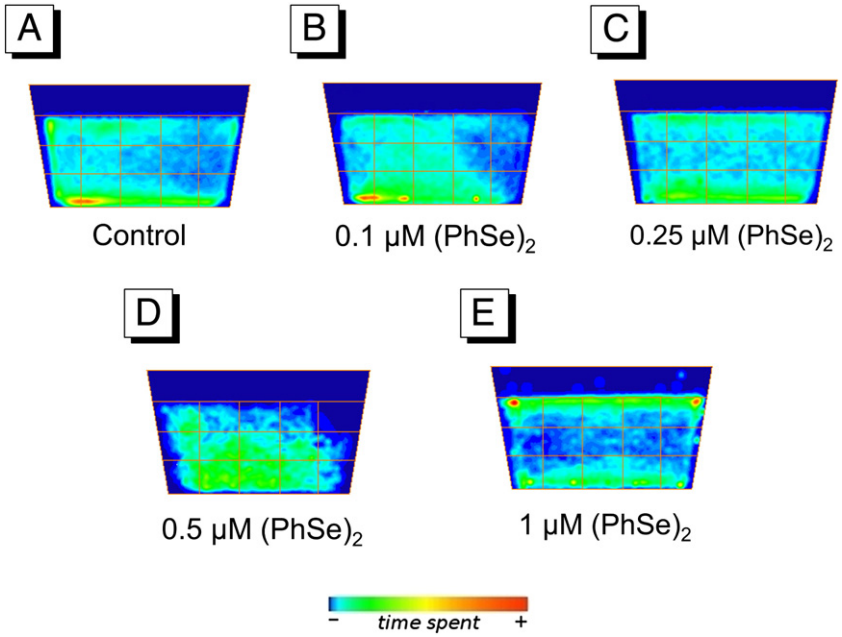

Fig. 4. Occupancy plot of exploratory activity and homebase formation in the novel tank. Occupancy plots of control (0.04% DMSO) (A) and 0.1 μM (B), 0.25 μM (C), 0.5 μM (D) and 1 μM $(PhSe)_2$ (E) groups displaying specific patterns of time spent in each segment of the apparatus during a 6-min trial. Increased time spent in a region indicates homebase formation. The data were analyzed using video-tracking software (ANY-maze, Stoelting CO, USA).

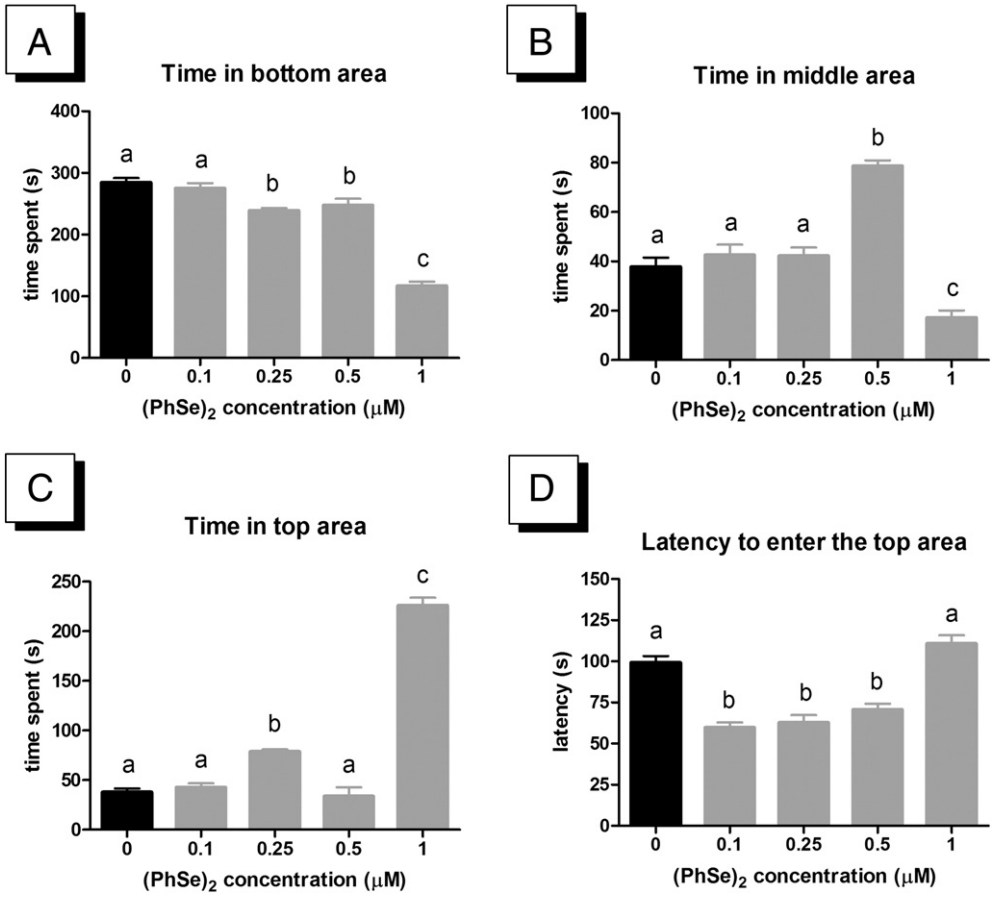

Fig. 5. Classical endpoint analysis for the assessment of anxiolytic-like behavior. The graph shows time spent at the bottom (A), middle (B), and top (C) as well as latency to the top (D) area. The data were analyzed by one-way ANOVA followed by Bonferroni's post hoc test, considering $p \leq 0.05$ as significant. Distinct letters indicate significant differences among the control (black) and $(PhSe)_2$ groups (gray).

(2007) and further validated by the pharmacological administration of anxiolytic/anxiogenic drugs (Stewart et al., 2010a, 2011; Wong et al., 2010). Furthermore, animals treated with 0.25 μM $(PhSe)_2$ explored the entire apparatus, performing more lateral activity at the top compared with the fish in the control groups. Typically, control fish entered the top area of the tank and rapidly returned to the bottom. Such an alteration in the exploration of the top area is also considered an indication of anxiolytic effects (Rosemberg et al., 2011, 2012). Fish treated with 0.25 μM $(PhSe)_2$ did not show a preference for any specific section of the apparatus. A recent debate about the preferential regions inside the novel tank led researchers to postulate the possible formation of a homebase in this apparatus (Stewart et al., 2010a). Recently, homebase behavior was defined as the tendency to establish a key safe location that the fish spends more time in and repeatedly returns to after exploring a novel environment (Kalueff et al., 2013). The occupancy plot analysis showed that animals treated with 0.1 μM $(PhSe)_2$ formed dispersed homebases, and complete abolishment of homebase formation was observed in animals exposed to 0.25 μM $(PhSe)_2$. The absence of a key safe location in the bottom area of the tank is associated with an increase in the time spent in the upper area, indicating the anxiolytic-like effect of $(PhSe)_2$ during a novel experience.

Despite the absence of homebase formation, animals treated with 0.25 μM $(PhSe)_2$ spent more time at the bottom of the tank. Similar results were observed in the fish exposed to drugs that evoke anxiolytic-like effects. Acute exposure to ethanol for 20 min led to increased exploration of the top area. However, in a 6-min trial, the animals showed a preference for the bottom, similar to what was observed with non-exposed fish (Rosemberg et al., 2012). Moreover, exposure of animals to 0.25 μM $(PhSe)_2$ for 30 min caused similar anxiolytic-like effects in this test compared to treatment with citalopram and desipramine (Sackerman et al., 2010), fluoxetine (Wong et al., 2010), olanzapine (Seibt et al., 2010), and nicotine (Levin et al., 2007; Stewart et al., 2010b). However, fish exposed to 1 μM $(PhSe)_2$ showed immobility, which was similar to what was observed in zebrafish exposed to diazepam (Mussulini et al., 2013), or lethargy, which was also observed in animals immersed in chlordiazepoxide. Reduced diving and bottom dwelling, which were also observed in fish treated with high doses of buspirone (Bencan et al., 2009), were noted as well. Although these side effects were observed in animal models after treatment with high concentrations of anxiolytic drugs, zebrafish treated with 1 μM $(PhSe)_2$ also displayed burst swimming, loss of innate preference for the bottom, and jumping behavior.

Selenium (Se) is an essential micronutrient for all vertebrate species, and it has a narrow margin between essentiality and toxicity in fish and vertebrates (Maher et al., 2010). Dietary selenomethionine, an organic selenium compound, showed a similar narrow margin for toxicity compared to inorganic forms (Thomas and Janz, 2011). Indeed, selenite in water was able to produce changes in the swimming performance of zebrafish (Massé et al., 2012). Therefore, the chaotic behavior shown by the animals exposed to 0.5 μM $(PhSe)_2$, the U-shaped curve observed in the latency to enter the top, and the episodic immobility and burst swimming observed in the 1 μM-treated group may be due to the potential toxicity of $(PhSe)_2$.

Because we aimed to verify that $(PhSe)_2$ demonstrates anxiolytic-like effects in zebrafish subjected to novelty stress, only the treated groups that did not show significant changes in swimming performance (distance traveled, episodes of immobility) were considered to provide support for this aim. Although animals exposed to 1 μM $(PhSe)_2$ spent

more time at the top, which is a remarkable characteristic observed in fish treated with anxiolytic compounds, this concentration clearly induced other side effects. Therefore, we assume that only $(PhSe)_2$ concentrations of 0.1 μM (lower latency to the top area and initial dispersal of homebase formation) and 0.25 μM (lower latency, more time spent at the top and abolishment of homebase formation) evoked anxiolytic-like behavior in zebrafish in a gradual manner. However, a full understanding of homebase abolishment and its use in anxiety drug screening requires further study. Therefore, the zebrafish appears to be an excellent animal model for testing the effects of organoselenium compounds due to the narrow margin between desirable pharmacological effects and toxic effects in fish. This characteristic can minimize the number and groups of animals needed in trials, which is an important consideration in the ethical debate concerning animal experimentation (Mandal and Parija, 2013).

5. Conclusion

This study demonstrates the potential anxiolytic effect of $(PhSe)_2$ during novelty stress using the novel tank diving test. Furthermore, we are the first to show the effects of different concentrations of $(PhSe)_2$ on zebrafish behavior. Because distinct models of pathologies (Alfaro et al., 2011; Braga et al., 2013; Lu et al., 2013; Song and Pimplikar, 2012; Stewart et al., 2013) and drug abuse (Kyzar et al., 2012; Maximino et al., 2011) have been studied using this species, our narrow exposure concentration curve can be used to evaluate the role of $(PhSe)_2$ as a protective compound in such situations. Finally, we hope that our data can provide researchers with an additional insights to further understand homebase behavior and its use in future anxiolytic drug discovery.

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